

Larval Development and Autogeny in *Ochlerotatus camptorhynchus* (Thomson) (Diptera: Culicidae) from Southern Victoria

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Larval development and autogeny was examined in the mosquito *Ochlerotatus camptorhynchus* (Thomson) from southern Victoria. Larvae of *Oc. camptorhynchus* were reared in the laboratory at 5 constant temperatures (15, 20, 25, 30 and 35°C) and three constant salinities (0, 18 and 36 ppK). Of the five temperatures, survival ranged from 35.6% at 35°C to 84.4% at 20°C, and development times ranged from 12.1 to 37.1 days at 35°C and 15°C respectively. The minimum threshold temperature for development was 7.3°C, and the thermal constant was 324.0 ± 12.8 SE degree-days. No differences in development times or survival were detected for the three salinities. Adult mosquitoes reared from field-collected pupae and larvae reared on a high-nutrition diet displayed no autogenous egg development. A positive relationship was found between adult body size (wing length) and fecundity in blood-fed adults, with fecundity ranging from 40 to 112 eggs per female.

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INTRODUCTION

The southern saltmarsh mosquito, *Ochlerotatus camptorhynchus* (Thomson), is an abundant mosquito species in southern coastal Australia from southwest Western Australia around to the southern coast of New South Wales, including Tasmania (Dobroworsky 1965; Russell 1993). It is a confirmed vector of Ross River virus (Ballard and Marshall, 1986) and suspected vector of Barmah Forest virus (Aldred et al. 1990; Russell 1995). *Ochlerotatus camptorhynchus* was also detected on the North Island of New Zealand during 1998 (Hearnden et al. 1999), and a large eradication program is now under way.

Larvae of *Oc. camptorhynchus* are typically found in brackish to fresh ground pools associated with coastal swamps and bushland (Dobroworsky 1965; Lee et al. 1984), but also in some salinity-affected areas inland (Wishart 2002). No studies have previously investigated the responses of immature stages of *Oc. camptorhynchus* to different temperatures or salinities.

Autogeny, or the ability to develop an initial batch

of eggs without a blood meal, has been described to varying levels in several Australian mosquito species including *Anopheles hilli* Woodhill and Lee (Sweeney et al. 1973), *Ochlerotatus vigilax* (Skuse) (Sinclair 1976; Hugo et al. 2003), *Culex annulirostris* Skuse (Kay et al. 1986), *Culex sitiens* Wiedemann (Fanning et al. 1992), *Ochlerotatus australis* (Erichson) (Brust 1997) and *Culex molestus* Förskal (Dobroworsky 1954). Of these mosquitoes, arguably the most closely related ecologically is *Oc. vigilax*, a saltmarsh mosquito replacing *Oc. camptorhynchus* as the dominant mosquito in northern coastal areas from southern New South Wales around to southern Western Australia (Lee et al. 1984; Russell 1993). Autogeny in *Oc. camptorhynchus* has not previously been investigated. Production of an autogenous egg batch may delay the first mosquito-host contact (Kay et al. 1986), and is thought to accelerate the rate of natural increase of mosquito populations (Tsuji et al. 1990).

The purpose of this study is to increase our understanding of the development and survival of larval *Oc. camptorhynchus*, which may provide for greater accuracy of timing of larvicide applications, as

well as a better understanding of the basic ecological parameters of this important mosquito species.

METHODS

Larval development

Thirty newly hatched larvae (1st instar) of *Oc. camptorhynchus* (<12hrs old) from a saltmarsh at Breamlea (144°35' East, 38°13' South), near Geelong in southern Victoria, were put into each of six translucent plastic containers with 300 ml of water (salinity = 18 ppK). Sets of six containers (total = 180 larvae) were then placed in a water bath for each of 15, 20, 25, 30 and 35°C. Larvae were fed ground K9® Gold Fish Food (Go-Pet Petcare Solutions) at rates of: 1st and 2nd instars = 0.12 mg/larva/day, 3rd instar = 0.48 mg/larva/day, 4th instar = 0.96 mg/larva/day. Any excess food was removed daily. Subsamples of 20 adult females were taken from each temperature treatment, and those had a single wing removed and measurement with a graticule eyepiece at 10x magnification from the wing tip (excluding the fringe) to the arculus (Harbach and Knight 1980).

To evaluate salinity tolerance, seawater (36 ppK) was collected from near Breamlea and used as stock solution, diluted equally with distilled water to produce a concentration of 18 ppK, and with distilled water being used for the 0 ppK concentration. Thirty newly hatched larvae, again collected from the Breamlea saltmarsh, were added to 300 ml of each concentration (2 replicates each). Concentrations were kept constant by adding distilled water daily to compensate for evaporation. A constant temperature of 25°C was maintained with the use of a water bath. Larvae were fed as described above.

Average development time and survival for all immature stages were calculated using frequency-weighted means, based on daily counts. Analysis of Variance with Student Newman-Keuls post-hoc tests (SPSS v11) was used to compare differences in total development times and survival to adulthood between treatments. The relationship between larval development times and temperature, plus wing length and temperature, was examined using least squares linear regression. The day-degrees (K) needed for development at each experimental temperature (T°C) and duration of development (t days) was calculated from $K = t(T - C)$, where C is an extrapolation of minimum temperature for development.

Autogeny

Autogeny was assessed in two categories of mosquito: 1) adults derived from field-collected

pupae, and 2) adults derived from larvae reared on a high nutrition diet. All larvae and pupae were taken from a field site at Breamlea flora reserve near Geelong in southern Victoria (144°35' East, 38°13' South, Fig 3.1).

The field-sourced pupae were collected from the same site at Breamlea during September 2002 and October 2003 and were allowed to emerge over a 24-hour period in a cage of approx 0.5m³. The laboratory-reared larvae were collected during October 2003 as first instar and reared with a high nutritional feeding regime at a daily rate of: 1st and 2nd instar = 0.16 mg/larva, 3rd instar = 0.64 mg/larva, and 4th instar = 1.28 mg/larva. The field-collected pupae and laboratory-reared larvae were kept in water taken from the field (36ppK) with salinity kept constant by topping up containers with distilled water. All emerged adult mosquitoes were given immediate access to 10% sucrose solution and males were not removed from the cages. Immatures and adults were kept at ambient laboratory temperatures (approx 20 ± 5°C). Ten days after emergence, all female mosquitoes were removed and cold anaesthetised before dissection. Ovaries were dissected and placed on slides with a saline solution for inspection of follicles at 200x magnification. Recording of stages of ovarian development was done with reference to Clements and Boocock (1984).

A separate sample of mosquitoes derived from pupae at Breamlea flora reserve (October 2003) was allowed to blood-feed three days after emergence. These mosquitoes were dissected seven days after the blood meal and their fecundity (number of eggs per female) recorded, as well as with wing length.

RESULTS

Larval development

At all temperatures tested, the first and fourth instars had the shortest and longest development times, respectively (Table 1). A significant difference between total development time and temperature was obtained (d.f. = 4, M.S. = 1313.06, F = 66.65, $P < 0.01$). Mean development was 12.1 ± 0.9 days at 35°C, and 37.1 ± 1.3 days at 15°C. Survival to adulthood was significantly different between the temperature treatments (d.f. = 4, M.S. = 205.55, F = 12.42, $P < 0.01$), with 84.4% survival at 20°C and 35.6% survival at 35°C. No difference was obtained for total immature development in different salinity treatments (d.f. = 2, M.S. = 2.99, F = 0.22, $P = 0.80$). No difference was obtained for immature survival between the salinity treatments (d.f. = 2, M.S. = 4.50, F = 1.50, $P = 0.35$).

Table 1 Duration, development rate and survival of immature stages of *Oc. camptorhynchus* at constant water temperatures and salinities. *Values followed by different letters are significantly different at the 5% level (Student-Newman-Keuls test). ^Proportion of immature development per day.

Tempera- ture °C	Days (mean ± SE) in each stage						Develop- ment rate^	%Sur- vival*
	I	II	III	IV	P	Total*		
15	4.1 ± 0.5	6.5 ± 1.0	8.1 ± 1.2	11.3 ± 1.2	7.1 ± 0.9	37.1 ± 1.3a	0.027	68.9 a,b
20	3.1 ± 0.8	4.2 ± 1.1	5.3 ± 1.2	7.5 ± 1.3	6.4 ± 1.2	26.5 ± 1.2b	0.038	84.4 a
25	2.4 ± 0.9	3.3 ± 1.0	4.0 ± 1.0	5.4 ± 1.1	5.1 ± 1.0	20.2 ± 1.0c	0.05	76.1a
30	1.8 ± 0.6	2.3 ± 0.7	2.6 ± 0.8	3.5 ± 1.0	3.1 ± 0.9	13.3 ± 0.9d	0.075	52.8 b
35	1.5 ± 0.6	2.3 ± 0.7	2.5 ± 0.7	28 ± 1.0	3.0 ± 0.8	12.1 ± 0.9d	0.083	35.6 c
Salinity (ppK) at 25°C								
0	2.5 ± 0.8	3.2 ± 1.0	4.1 ± 1.0	5.2 ± 1.1	4.0 ± 0.9	18.9 ± 1.1a	0.052	98.5 a
18	2.4 ± 0.8	3.2 ± 0.9	4.2 ± 1.0	5.2 ± 1.1	5.0 ± 1.0	20.0 ± 1.0a	0.05	88.3 a
36	2.5 ± 0.8	3.2 ± 1.0	4.1 ± 1.0	5.5 ± 1.1	4.5 ± 1.0	19.9 ± 1.0a	0.053	93.3 a

Table 2. Percentage ovarian development in two categories of adult female *Oc. camptorhynchus*, 10 days post emergence.

Source of adults	Percent ovarian stage									Total No.
	Ia	Ib	Iia	lib	IIIa	IIIb	Iva	Ivb	V	
Pupae taken from field	0	19	51.2	26.2	3	0.6	0	0	0	168
Larvae reared on high diet	0	12.4	42.8	32.4	9	3.4	0	0	0	145

A significant linear relationship (d.f. = 1, M.S. = 0.01, F = 109.01, P < 0.01) was obtained between water temperature and rate of development with a coefficient of determination of R² = 0.971 (Fig 1). The lower threshold for development was extrapolated to 7.3°C, and the thermal constant required for complete development was calculated as 324.0 ± 12.8 SE degree-days. A significant negative linear relationship (d.f. = 1, M.S. = 1.12, F = 68.133, P < 0.01) was obtained for wing length and temperature (Fig 2.)

Autogeny

No autogeny was observed in either category of *Oc. camptorhynchus* assessed in this study. The most common stage of follicular development in the two categories examined was stage IIa (field = 51.2%, laboratory = 42.8%) (Table 2). The next highest percentage of follicular development was stage IIb (field = 26.2%, laboratory = 32.4%). All blood fed mosquitoes developed eggs to stage V, seven days after the blood meal. A significant linear relationship

was found between body size (wing length) and the number of mature follicles per mosquito (d.f. = 1, MS = 5,668.00, F = 41.64, P < 0.01) (Fig 3). Fecundity of blood-fed females ranged from 40 to 112 eggs per mosquito (72.41 ± 2.99 SE, n = 34).

DISCUSSION

This study demonstrates that *Oc. camptorhynchus* is well adapted to cooler temperatures and is widely tolerant of different salinities. In contrast to *Oc. vigilax*, the Breamlea population of *Oc. camptorhynchus* exhibits no autogeny, indicating a different survival strategy to its more northern congener.

The development of larval *Oc. camptorhynchus* responded to temperature and was linear between 15 and 35°C. None of the temperatures tested were lethal to the Victorian strain *Oc. camptorhynchus*. The development threshold temperature of 7.3°C for *Oc. camptorhynchus* suggests development of immatures

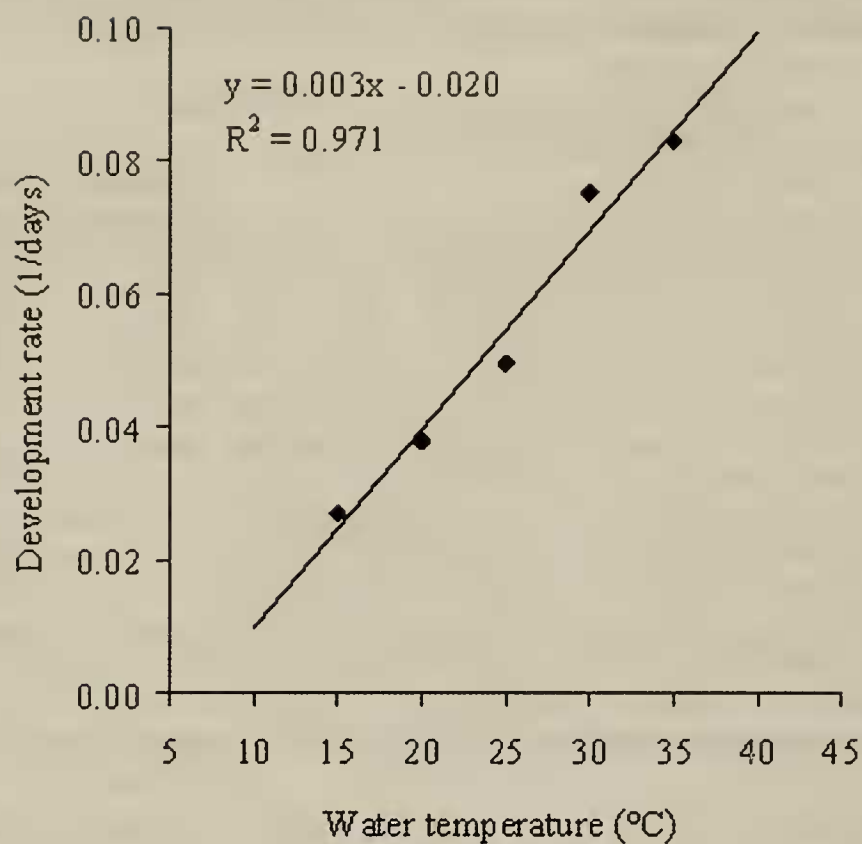
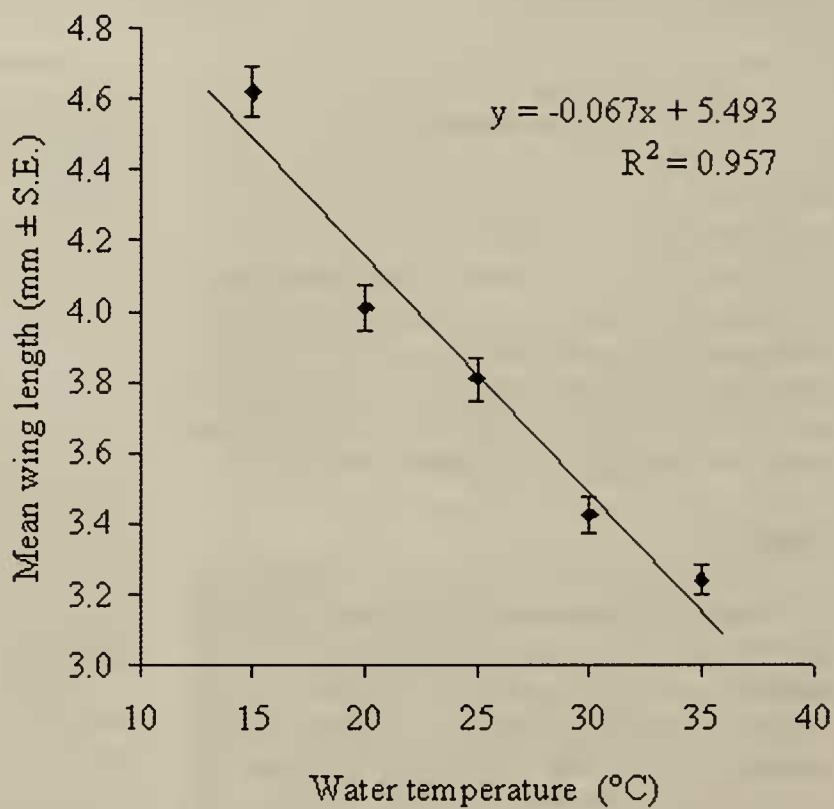


Figure 1. Relationship between development rate of immature stages of *Oc. camptorhynchus* and five constant water temperatures.

Figure 2. Relationship between wing length of adult female *Oc. camptorhynchus* and five constant water temperatures.



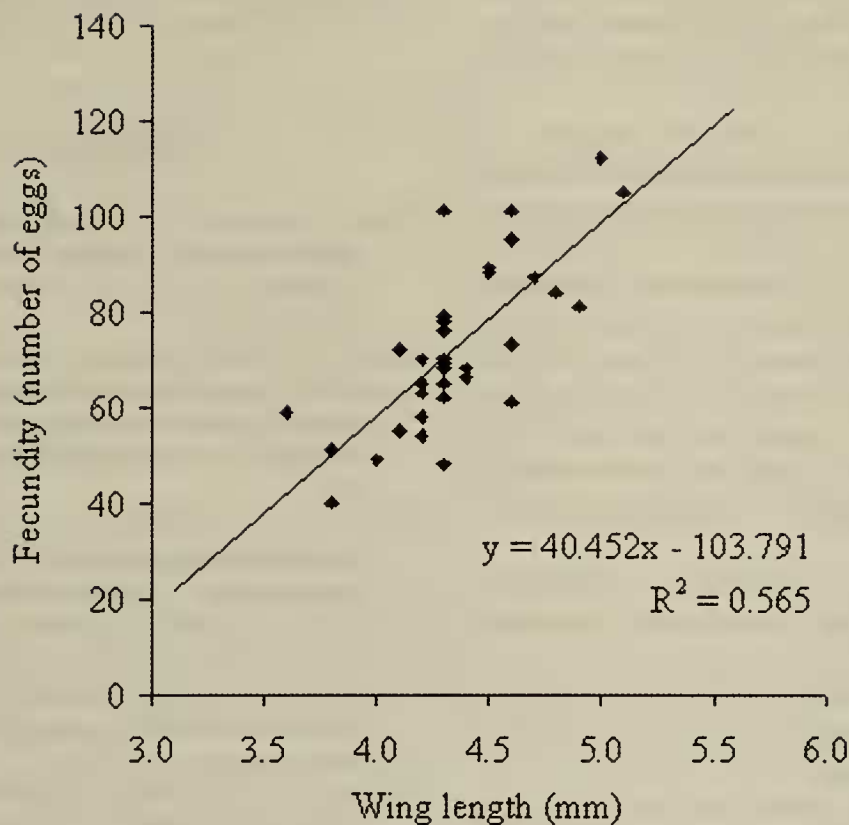


Figure 3. Relationship between wing length and fecundity for blood-fed *Oc. camptorhynchus* (n = 34).

will continue in winter in southern Victoria, where minimum temperatures of approximately 8°C commonly occur (Bureau of Meteorology 1993). The larvae of *Oc. camptorhynchus* at 15°C had higher survival (69%) and a lower development threshold (7.3°C) compared to more northern species reared at 15°C, such as *Cx. sitiens* from southeast Queensland (10% survival, 11.9°C threshold) (Mottram et al. 1994), *Cx. annulirostris* from northern Victoria (3 % survival, 9.7°C threshold) (McDonald et al. 1980) and *Ae. aegypti* from north Queensland (24% survival, 8.3°C threshold) (Tun-Lin et al. 2000).

At 25°C, the absence of any affect of salinity on the development or survival of larval *Oc. camptorhynchus* suggests a high level of adaptation to saline conditions. The mechanism for survival in high salinities is likely to be a capacity to produce non-toxic osmolytes (Bradley 1987), which can neutralise the harmful effects of high salt concentrations. Possible interaction between salinity and temperature was not examined and differences in survival and/or development may be observed at varying salinities at higher or lower temperatures.

During expected spring or summer temperatures of 15-25°C, and following inundation of larval habitat, survival of *Oc. camptorhynchus* will be high, depending on predation, and development may take 20-37 days. No threshold salinity, above which survival is curtailed, is apparent for *Oc. camptorhynchus* between 0ppK and 36ppK. Given the optimal stage for treatment with the control agent *Bacillus thuringiensis israelensis* are 2nd to 3rd instars, this suggests a large treatment window of approximately 7-15 days, and if s-methoprene were to be used, the time before treatment could be extended to 4th instar if necessary. However, in situations where prolonged temperatures of above 30°C occur, survival of *Oc. camptorhynchus* might be expected to be lower, and control may not be warranted. The operational criteria for control, therefore, should be based on larval densities, size of breeding site and on proximity to residential areas.

Adult *Oc. camptorhynchus* derived from field-collected pupae and larvae reared with high nutrition in the laboratory both failed to exhibit autogeny. This differs remarkably compared to the saltmarsh

mosquitoes *Oc. vigilax* from Australia (Sinclair 1976; Hugo et al. 2003), and *Oc. taeniorhynchus* from the USA (O'Meara and Edman 1975), where autogeny rates of up to 100% and 94.4% were observed in these studies, respectively.

Of all the adults assessed for autogeny, the majority of displayed ovarian development to stages IIa or IIb, which is the previtellogenic resting stage described by Clements and Boocock (1984). At this developmental stage, it is thought that a blood meal is required to facilitate further follicular development and oviposition. That a greater percentage of adults reared on a high diet had follicles over stage IIb (12.4%) compared to adults derived from field-collected pupae (3.6%) suggests that nutrition may be important in influencing ovarian development in *Oc. camptorhynchus*.

The fecundity of blood-fed *Oc. camptorhynchus* increased with body size, a well-known relationship documented with other mosquitoes (Nayar and Sauerman 1975; Armbruster and Hutchinson 2002). Larger mosquitoes might therefore be expected to produce larger egg batches.

The anautogeny apparent from the sampled *Oc. camptorhynchus* suggests this species may require blood meals for survival and egg development and therefore appetential dispersal after emergence might occur earlier, in relative terms, than for *Oc. vigilax*, as time for egg development and oviposition is not required. This in turn suggests that the role of *Oc. camptorhynchus* in biological transmission would commence earlier, as this species is unlikely to mature an autogenous egg batch. From an ecological standpoint, dispersal of anautogenous *Oc. camptorhynchus* would seem to be a risky survival strategy when suitable saltmarsh habitats are discontinuous, as on the Bellarine Peninsula where this study was performed. This result might be expected to change geographically, where different genetic and environmental factors may influence autogenous expression (Sota and Mogi 1995; Hugo et al. 2003), and is worthy of further study.

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